TAPERED MICROFLUIDIC DEVICE FOR MULTI-PARTICLE SEPARATION BASED ON SEDIMENTATION PRINCIPLE

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A thesis submitted in fulfilment of the
requirements for the award of the degree of
Doctor of Philosophy (Electrical Engineering)

Faculty of Electrical Engineering
Universiti Teknologi Malaysia

DECEMBER 2017

Specially dedicated with love and affection to:

my parents Ahmad Shapii & Aslinah Masran,
parents in laws & siblings

my great hearted husband Redzuan Shah Yussoff
and lovely children
Aryana Safiyyah, Aariz Affan, Ahsan Arrazi & Aafia Amanda

thank you for all of your supports along the way
you mean world to me, who is indeed a treasure from the Lord

May Allah (swt) shower his blessings upon all of you

ACKNOWLEDGEMENT

First and foremost, I would like to express my heartily gratitude to my supervisor, Assoc. Prof. Ir. Dr. Mohd Ridzuan Bin Ahmad for the guidance and enthusiasm given throughout the progress of this project.

I would like to thank to Micro-Nano Systems Engineering Research Group (MNSERG) members; Amelia, Asraff, Emma, Diya, Habib, Salma, Siti Nadia and Hafiz for their cooperation and helps in this project. My appreciation also goes to members of Robotic and Instrumentation Lab; Amirah, Sarah, Marwan, Abdul Rahman, Veni, Umar and Goh for their support and encouragement.

I am grateful for the opportunity given by Micro-Nano Control Engineering Bio-Robotics Lab at Nagoya University Japan for three months research collaboration attachment program in 2015. I am thankful for the various exposures of research environments, fruitful discussions and close supervision from sensei: Prof. Yasuhisa Hasegawa, Assistant Prof. Masahiro Nakajima and Assistant Prof. Masaru Takeuchi. Undeniably, it was truly an unforgettable experience.

Above ground, I am indebted to Universiti Tun Hussein Onn Malaysia (UTHM) and Ministry of Higher Education (MOHE) for the sponsorships given throughout the course of this study.

Nevertheless, my great appreciation dedicated to my friends and those whom involved directly or indirectly with this project.

ABSTRACT

This thesis presents a label-free tapered microfluidic device for a passive multi-particle separation. Separation process plays a significant role in various industries for example, biomedical diagnostic, food processing and substance purification. The growing needs for continuous separation process lead to the creation of numerous microfluidic based separation devices. Currently, microfluidic based separation devices are associated with limitations in terms of design complexity, sample purity and separation throughput. Therefore, a simple novel passive tapered microfluidic separation device with various taper angles (6°, 12°, 20° and 25°) is proposed for high purity separation of biological and non-biological samples. The device utilizes coupling mechanism between hydrodynamic separations along with sedimentation effect for enhancement of sample purity. Computer-aided design software was employed during design stage while Finite Element Analysis (FEA) software was used for device design's optimization. The device was fabricated using a soft lithography technique and was characterized in terms of physical dimensions and leakage conditions. Size based separation simulations using FEA were carried out for 3 µm and 10 µm diameters polystyrene (PS) microbeads samples as well as a mixture of 3 µm PS microbeads and Human Cervical Epithelial Carcinoma (HeLa) cells. Through FEA simulations, larger particles were collected at Outlet 1 and small particles were collected at Outlet 2 using 20° and 25° tapered devices. Furthermore, experimental tests were conducted with similar settings and samples as in the simulations. Successful multi-particle separations were observed using 20° and 25° tapered devices at 0.5 to 3.0 µl/min flow rates. These results were in agreement with simulation results obtained. Highest purity of 98% was achieved for both samples with the use of 3.0 µl/min flow rate. As a conclusion, a passive tapered microfluidic device capable of multi-particle separation at high sample purity was developed.

ABSTRAK

Tesis ini membentangkan peranti *microfluidic* tirus bebas label untuk pengasingan pelbagai zarah secara pasif. Proses pengasingan memainkan peranan penting dalam pelbagai industri sebagai contoh diagnostik bioperubatan, pemprosesan makanan dan penulenan bahan. Peningkatan keperluan bagi proses pengasingan secara berterusan membawa kepada penciptaan pelbagai peranti pengasingan berasaskan *microfluidic*. Pada masa ini, peranti pengasingan berasaskan *microfluidic* dikaitkan dengan pembatasan daripada segi kerumitan reka bentuk, ketulenan sampel dan jumlah lepas pengasingan. Oleh itu, sebuah peranti *microfluidic* tirus yang baharu dan ringkas dengan pelbagai sudut tirus (6°,12°, 20° and 25°) dicadangkan bagi tujuan pengasingan berketulenan tinggi bagi sampel biologi dan bukan biologi. Peranti ini menggunakan mekanisme gandingan antara pengasingan hidrodinamik dan kesan pemendapan bagi peningkatan ketulenan sampel. Perisian reka bentuk bantuan komputer telah digunakan semasa peringkat reka bentuk manakala perisian *Finite Element Analysis* (FEA) telah digunakan untuk pengoptimuman reka bentuk peranti. Peranti telah difabrikasi menggunakan teknik litografi lembut dan pencirian dari segi dimensi fizikal serta keadaan kebocoran. Simulasi pengasingan berdasarkan saiz telah dilakukan untuk manik mikro polisterina (PS) berdiameter 3 μm dan 10 μm dan juga campuran manik mikro polisterina 3 μm bersama sel Human Cervical Ephitelial Carcinoma (HeLa). Melalui simulasi, partikel besar telah dikumpulkan di *Outlet* 1 dan partikel kecil telah dikumpulkan di *Outlet* 2 menggunakan peranti tirus bersudut 20° dan 25°. Ujian ujikaji telah dilakukan menggunakan sampel dan pelarasan yang sama seperti simulasi. Pengasingan pelbagai zarah berjaya diperhatikan menggunakan peranti bersudut tirus 20° dan 25° pada kadar aliran 0.5 hingga 3.0 μl /min. Keputusan ini adalah sepadan dengan hasil simulasi yang diperolehi. Ketulenan tertinggi sebanyak 98% telah dicapai untuk kedua-dua sampel dengan menggunakan kadar aliran 3.0 μl /min. Sebagai kesimpulan, sebuah peranti *microfluidic* tirus pasif yang mampu memisahkan pelbagai zarah pada ketulenan sampel yang tinggi telah dibangunkan.

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LIST OF ABBREVIATIONS

μTAS	-	Micro Total Analysis System
3D	-	Three Dimensional
ABPS	-	Acoustic Band Pass Particle Sorter
AC	-	Alternating Current
As-PFF	-	Asymmetric Pinch Flow Fractionation
C3D10	-	Quadratic Tetrahedral Element
CEL	-	Coupled Eulerian-Lagrangian
CF	-	Contrast Factor
CM	-	Claussius-Mossotti
CO_2	-	Carbon Dioxide
CTCs	-	Circulating Tumour Cells
DC	-	Direct Current
DEP	-	Dielectrophoresis
DLD	-	Deterministic Lateral Displacement
DLW	-	Direct Laser Writing
EC3D8R	-	Eulerian Material
Eos	-	Equation of State
EWOD	-	Electrowetting dielectric
FACS	-	Fluorescent Activated Cell Sorter
FEA	-	Finite Element Analysis
FEM	-	Finite Element Method
FFF	-	Field Flow Fractionation
GPL	-	General Public License
GUI	-	Graphical User Interface
HeLa	-	Human Servical Epithelial Carcinoma
HOT	-	Holographic Optical Tweezers
IAMS	-	Integrated Acoustic Magnetic Separator

IDS	-	Iso Dielectric Separation Dielectrophoresis
IDT	-	Interdigitated Transducers
IPA	-	Isopropyl alcohol
Jurkat	-	Acute T Cell Leukaemia
LoC	-	Lab on a Chip
MACS	-	Magnetic Activated Cell Sorter
MT-MACS	-	Multi Magnetic Activated Cell Sorter
MVM	-	Microvortex Manipulation
n-DEP	-	Negative Dielectrophoresis
O_2	-	Oxygen
OC	-	Optical Chromatography
ODEP	-	Optical Dielectrophoresis
OET	-	Lateral-Field Optoelectronic Tweezers
OET	-	Optoelectronic Tweezers
OPFF	-	Optically Enhanced PFF
p-DEP	-	Positive Dielectrophoresis
PDMS	-	Polydimethylsiloxane
PFF	-	Pinch Flow Fractionation
PS	-	Polystyrene
RBC	-	Red Blood Cells
SAW	-	Standing Acoustic Wave
SPLITT	-	Split-Flow Lateral-Transport Thin
SS-MOFF	-	Single Stage Multi-Orifice Flow
TwDEP	-	Travelling Wave-DEP
USD	-	United States Dollar
USW	-	Ultrasonic Standing Wave
UV	-	Ultraviolet
WBC	-	White Blood Cells

LIST OF SYMBOLS

ρ_d	-	Particle Density
(U_{sed})	-	Sedimentation Velocity
c_0, s, γ	-	Equation of State Constants
F_L	-	Lateral Force
U_P	-	Particle Velocity
U_S	-	Shock Velocity
C_1	-	Material Constant
C_{10}, D_1	-	Neo-Hookean Material Parameters
D_h	-	Hydraulic Diameter
H_{in}	-	Entrance Width of The Separation Channel.
I_1	-	First Invariant of The Left Cauchy-Green Deformation Tensor
R_h	-	Hydrodynamic Resistance
U_p	-	Particle Velocity
τ_f	-	Flow Characteristic Time
τ_r	-	Particle Relaxation Time
ΔP	-	Pressure Difference
Δp	-	Density Difference
$\sqrt{A_c}$	-	Characteristic Length
μ	-	Viscosity
A	-	Cross Sectional Area
F_b	-	Buoyancy Force
F_d	-	Drag Force
F_s	-	Sedimentation Force
h	-	Height
P, p	-	Pressure

Re	-	Reynolds Number
St	-	Stokes Number
C	-	Aspect Ratio
I	-	Current
L	-	Linear Dimensions
P	-	Perimeter
Q	-	Flow Rate
R	-	Resistance
U	-	Average Fluid Velocity
V	-	Voltage
a,b,ω_0 ω_1	-	Widths
d	-	Particle Diameter
f	-	External Forces
g, a	-	Gravitational Acceleration
r	-	Radius of The Particle
v	-	Velocity Vector
η	-	Fluid Dynamic Viscosity.
κ	-	Bulk Modulus
μ	-	Shear Modulus
ρ	-	Fluid Medium Density

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Separation process is essential for a wide range of technologies in both industries as well as research. It can be defined as a process to select or removal of specific impurities from heterogeneous mixture. Separation or sorting of micro-particles and droplets based on physical characteristics is important in numerous applications such as diagnostics, biological analyses, food industries and chemical processing. In food industries, separation of harmful particles and bacterial growth can be monitored [1]. Generally, the separation module is crucial in preparing samples for further analysis. In medical diagnostic, physical properties of cells will be investigated for example size, type, density and stiffness to classify the specific population of interest. Several lines of evidence suggested that cancerous cells tend to be softer than healthy cells [2], while malaria infected red blood cells will become stiffer and more rigid [3]. Cell-free plasma is required for diagnosis related to blood-bourne cancer biomarkers [4]. Purified platelets are used extensively during clinical surgery and recovery [5]. Cell separation is used for specific cell population enrichment intended for diagnostic and therapeutic applications [6].

A separation unit or module can be placed either upstream or downstream in an application depending on its purpose. The efficiency of separation process can be evaluated by using several indicators like sample purity, enrichment and throughput [7]. High purity relay information regarding concentration of particular samples. Enrichment is the enhancement of target sample as compared to the background sample indicates selectivity of separation process. Throughput relates the separation

speed typically reported in volumetric flow rate or number of samples per minute. Figure 1.1 below shows the conceptual frameworks for a separation system.

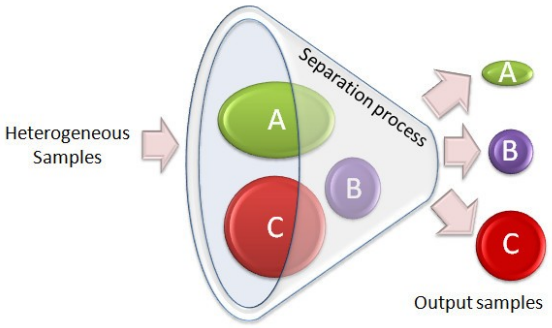

Figure 1.1: Conceptual frameworks for a separation system. Heterogeneous samples are collected separately at the outlets.

1.1.1 Conventional Separation Techniques

Conventional separations approaches use physical descriptors for examples size, shape and density (filtration, centrifugation) or affinity based approach (flow cytometry, fluorescent activated cell sorter (FACS) and magnetic activated cell sorter (MACS)). In size based filtration, the filter used is designed to comply with shape and size of the particles of interests. Density based centrifugation technique works using buoyant gravity of particles is more popular both in clinical and laboratories applications for examples blood sampling. However, problem with cell homogeneity still persists and becomes significant for cell populations with very small density differences. They rely heavily in filtration approach and usually adopted bulky equipments and labor intensive. Labelling or tagging used for biological separations obviously is expensive and requires delicate handling [1], [8]. Furthermore, not all fluorescent markers are suitable to be used by cells, specific requirements and knowledge are required to understand how the binding between fluorescent marker and protein of interest can be established. Various limitations of conventional techniques contributed to the innovation of various label-free techniques for simple and efficient separation at a lower cost. Label-free is a coined term to represent the use of certain physical attributes of particles of interest which can be used for efficient separations. Some of famous attributes are size, density, deformability and stiffness.

1.1.2 Microfluidic Technologies for Particles Separations

Recent trends towards label-free separation approach is widely accepted enabling the birth of microfluidic technology. Microfluidic devices have emerged as multi-functional and powerful platform for separation ranging from nano-micro sized particles to biological cells. The evolutions of microfabrication techniques enabling the research involve micron-sized particles. These advancements allow for creation of microchannels which work on very small platform (100 nm to 500 μm) and employ unique characteristics of microscale flow which are named microfluidic devices [9]. Extensive reviews have been presented by other researchers to highlight on several benefits of utilizing microfluidic as given by [10]–[16]. Microfluidic consumes fewer reagents, uses smaller sample volume (typical clinical or analytical sample volumes (~0.1–10 ml) and the internal volume of microfluidic chips (~0.01–10 μl)) [17], cost effective with less power usage [18]. Other than that, microfluidic usage can minimize operator handling, perform faster and continuously while producing reliable results [19]. The benefits of miniaturization paved the way for integration of separation module into a complete lab on chip (LOC) device and supporting the creation of Micro Total Analysis System (μTAS). Comprehensive reviews on these benefits can be found from [20]–[23]. Table 1.1 compares these available technologies.

Table 1.1: Comparison between conventional and microfluidic based separation device.

Requirement	Conventional	Microfluidic Based
Label	Yes	Flexible
Device size	Big and bulky (benchtop)	Small and portable
Device complexity	High	Low
Processing mode	Batch	Continuous
Operator handling	High	Minimum

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